How many trimers? Modeling influenza virus fusion yields a minimum aggregate size of six trimers, three of which are fusogenic: Supplementary information

A PABM model of the in vitro virus fusion setups

The following PABM model represents the steps observed in in vitro virus fusion setups:

\[ HA \equiv (lx_{FS}.lt_{LC}.lx_{FP}.(x_{f,b}^j.\varnothing x_{agg,b}^j + x_{b}^j.\varnothing x_{agg}^j).lx_{SA}; -) \]  

(1)

\[ SA \equiv (mxt_{FS}.xl_{LC}.lx_{FP}.lx_{f,f,b}.lx_{agg/agg,b}^j.\varnothing x_{SA}; -) \]  

(2)

where \( i \), \( j \), \( k \) and \( l \) are the user-defined requirements for \( minHA_{Bound,Fusogenic} \), \( minHA_{Bound,Aggregate} \), \( minHA_{Free,Fusogenic} \) and \( minHA_{Free,Aggregate} \), respectively. The reacting systems are defined as:

\[ [HA^n] \] Virus content \( \circ \) \[ [SA^y] \] Cell content \( \]  

(3)

where \( x \) corresponds to the number of HA molecules and \( y \) corresponds to the number of SA molecules at the contact area between the virus and the cell. The only first step possible would be an interaction (binding) between the virus and the target membrane through the channel \( x_{SA} \), resulting in the following configuration:

\[ HA_b \equiv (lx_{FS}.lt_{LC}.lx_{FP}.x_{f,b}^j.\varnothing x_{agg,b}^j; -) \]  

(4)

\[ [HA_b]HA^{n-1}_a \] Virus content \( \circ \) \[ [mxt_{FS}.xl_{LC}.xl_{FP}.xl_{f,f,b}.lx_{agg/agg,b}^j]SA^{y-1} \] Cell content \( \]  

(5)

Note that the parallel composition of \( HA_b \) and \( HA \) permits a choice between an aggregation reaction involving a b or a f HA. An aggregation reaction involving \( HA_b \) would yield an \( HA'_b \) in the form of:

\[ HA'_b \equiv (lx_{FS}.lt_{LC}.lx_{FP}.x_{f,b}^j.\varnothing x_{agg,b}^{n-1}; -) \]  

(6)

whereas an aggregation reaction involving f HA would yield an \( HA' \) in the following form:

\[ HA' \equiv (lx_{FS}.lt_{LC}.lx_{FP}.x_{f,b}^j.\varnothing x_{agg,b}^{n-1}; -) \]  

(7)

Aggregate formation strictly precedes any conformational change, given that HA molecules that undergo this conformational change prior to aggregation are inactivated. After \( j+l \) aggregation reactions, the reacting systems will now have the following form:

\[ HA_{f,b} \equiv (lx_{FS}.lt_{LC}.lx_{FP}.x_{f,b}^j; -) \]  

(8)

\[ HA_{f,f} \equiv (lx_{FS}.lt_{LC}.lx_{FP}.x_{f,b}^j; -) \]  

(9)

\[ [HA_{f,b}]HA_{f,f}[HA^{n-1}_a] \] Virus content \( \circ \)  

\[ [(mxt_{FS}.xl_{LC}.xl_{FP}.xl_{f,f,b})^{n+1}]SA^{y-(j+1)} \] Cell content \( \]  

(10)

\(^1\)The notations are based on the syntax reported in M. David, J. Bantang and E. Mendoza, Transactions on Computational Systems Biology XI, 2009, 164 - 186.
Given that $j + l > i + k$, it is certain that the $i \varnothing x_{f,b}$ and $k \varnothing x_f$ actions in $HA_{f,b}$ and $HA_{f,f}$ will be executed. The first fusion pore (FP) can be formed between two systems in the following state:

$$HA_{f,b} = HA_{f,f} \equiv \langle !x_{FS},!x_{LC},!x_{FP}; \rangle$$

(11)

which can be alternately written as:

$$\langle !x_{FS},!x_{LC},!x_{FP}; \rangle HA^{x-(j+l)} \equiv \langle !x_{FS},!x_{LC},!x_{FP}; \rangle$$

(12)

The lipid channel (LC) can then be formed:

$$\langle !x_{FS},!x_{LC}; \rangle HA^{x-(j+l)} \equiv \langle !x_{FS},!x_{LC}; \rangle$$

(13)

Finally, the formation of the fusion site (FS) leads to membrane and content mixing of the two systems:

$$\langle !x_{FS}; \rangle HA^{x-(j+l)} \equiv \langle !x_{FS}; \rangle$$

(14)

Note that although the reactions in an in vitro fusion setup do not require the representation of compartments, a PABM model could be easily expanded to the in vivo scenario where reactions 7 to 16 occur inside the endosome. The representation of content mixing between the virus and the cell would also be straightforward in the sense that no additional variables representing the location of the contents would be needed.
B PRISM model

module fusion
virus_fp : [0..VIR] init 0;
virus_lc : [0..VIR] init 0;
virus_fused : [0..VIR] init 0;

/fusogenic HA
ha_b : [0..VIR*HA_f] init 0;
ha_f : [0..VIR*HA_f] init VIR*HA_f;
complex : [0..VIR*SA] init 0;
ha_ba: [0..VIR*HA_f] init 0;
ha_bc : [0..VIR*HA_f];
ha_fc : [0..VIR*HA_f];

//SA
sa_f : [0..SA] init SA;
sa_b : [0..SA] init 0;
x: [0..14] init 0;
[time] x<LONGWAIT -> (x'=min(x+1,14));

//binding reactions
[binding] ha_f>0&ha_b<(VIR*HA_f)&sa_f>0&sa_b<SA&
( (ha_f-1)>=0&(ha_b+1)<=HA_f*VIR&(sa_f-1)>=0&(sa_b+1)<=SA
b*ha_f*sa_f : (ha_f'=ha_f-1)&(ha_b'=ha_b+1)&
(sa_b'=sa_b+1)&(sa_f'=sa_f-1)&(complex'=complex+1);

//associated virus binding reactions
[unbinding] ha_b>0&(ha_b-1)>=0&sa_b>0&(sa_b-1)>=0&
ha_f<=(VIR*HA_f)&(ha_f+1)<=(VIR*HA_f)&(sa_f+1)<=SA&
complex>0&(complex-1)>=0
ub*complex : (ha_b'=ha_b-1)&(sa_b'=sa_b-1)&(sa_f'=sa_f+1)&
( ha_f'=ha_f+1)&(complex'=complex-1);

//aggregation can only occur after something is b; reactions must be coupled
[aggregation_f] x=LONGWAIT&ha_b>0&ha_f>0&(ha_fa+1)<=HA_f*VIR&(ha_f-1)>=0
ka*ha_f : (ha_f'=ha_f-1)&(ha_fa'=ha_fa+1);

[aggregation_b] x=LONGWAIT&ha_b>0&(ha_ba+1)<=HA_f*VIR&(ha_b-1)>=0
ka*ha_b : (ha_b'=ha_b-1)&(ha_ba'=ha_ba+1);

//conformational change
[conformational_change_b] ha_ba>0&min_b_agg&
( ha_b-1)=0&(ha_bc+1)<=HA_f*VIR
kf*ha_ba/factor : (ha_bc'=ha_bc+1)&(ha_ba'=ha_ba-1);

[conformational_change_f] ha_fa>0&min_f_agg&
( ha_f-1)=0&(ha_fc+1)<=HA_f*VIR
kf*ha_fa : (ha_fc'=ha_fc+1)&(ha_fa'=ha_fa-1);

[ffp] virus_fp=0&virus_lc=0&ha_bc>min_b_cc & ha_fc> min_f_cc & (virus_fp+1)<=VIR
fp : (virus_fp'=virus_fp+1);

[lc] virus_fp=1 & (virus_lc+1)<=VIR&(virus_fp-1)=0
lc : (virus_lc'=virus_lc+1) &(virus_fp'=virus_fp-1);
endmodule

rewards "VIRUS_FP"
true : virus_fp;
endrewards

rewards "VIRUS_LC"
true : virus_lc;
endrewards

CSL property checking:

const double t;
R("VIRUS_FP")=? [ I=t ]
R("VIRUS_LC")=? [ I=t ]